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Determination of the absolute configuration of an EAG active component in the sex pheromone gland of *Semiothisa cinerearia* Bremer *et* Grey (Lepidoptera: Geometridae)

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Abstract: The Chinese scholar tree looper, *Semiothisa cinerearia* Bremer *et* Grey (Lepidoptera: Geometridae), is the major defoliator of the Chinese scholar tree *Sophora japonica* L. in China. The elucidation of the absolute chemical structure of the sex pheromone component of *S. cinerearia* may provide a potential chemical for the pest control against the *S. cinerearia* infestations in the urban area. In this study, two sex pheromone gland compounds, *i. e.*, (6*Z*, 9*Z*)-6, 9-*cis*-3, 4-epoxy-heptadecadiene (6*Z*, 9*Z*-*cis*-3, 4-epoxy-17: H) and (3*Z*, 6*Z*, 9*Z*)-3, 6, 9-heptadecatriene (3*Z*, 6*Z*, 9*Z*-17: H), were detected in a 100∶4.8 ± 1.3 ratio ($N = 12$) during analyses of solvent extracts from virgin female *S. cinerearia* (2 – 3 d old) by comparison of their gas chromatography (GC) retention time and mass spectra (MS) with those of synthetic standards. Furthermore, the absolute configuration of 6*Z*, 9*Z*-*cis*-3, 4-epoxy-17: H in the sex pheromone gland of *S. cinerearia* was determined as (6*Z*, 9*Z*)-3*R*, 4*S*-epoxy-heptadecadiene by using a chiral capillary column (CycloSil-B, 30 m × 0.25 mm × 0.25 μm film) GC under the optimized oven temperature program. The mixture of the two synthetic enantiomers of 6*Z*, 9*Z*-3*R*, 4*S*-epoxy-17: H and 6*Z*, 9*Z*-3*S*, 4*R*-epoxy-17: H in a ratio of 1.28∶1 was injected into the female extracts, and thus the ratio was changed to 1.55∶1. Based on this analysis, the absolute configuration of the gland component 6*Z*, 9*Z*-*cis*-3, 4-epoxy-17:H was further confirmed as 3*R*, 4*S*. It is anticipated that control of *S. cinerearia* infestation with enantiomerically pure materials will be much more effective.

Key words: *Semiothisa cinerearia*; sex pheromone; (6*Z*, 9*Z*)-3*R*, 4*S*-epoxy- heptadecadiene; absolute configuration; GC-MS; chiral separation

1 INTRODUCTION

The scholars tree looper, *Semiothisa cinerearia* Bremer *et* Grey (Lepidoptera: Geometridae), is the most important defoliator of the Chinese scholar tree *Sophora japonica* L., which is advocated as one of the major trees for planting in Hebei, Henan, Shandong, Zhejiang, Jiangsu, Jiangxi and portions of several other Chinese provinces (Xiao, 1991). In north China, three to four generations of *S. cinerearia* can develop in a single year. Flights of adults begin in April/May and females lay eggs on the leaves, petioles, and young shoots. Each female lays an average of 420 eggs, with a maximum of 1 520. The first generation of larvae usually occur in early May and, the larvae feed very intensively in late May, mid-July, and early September, respectively. The larvae descend to the soil in September/October and overwinter as pupae (Xiao, 1991). Significant control efforts against *S. cinerearia* (mainly aerial treatment with chemical and bacterial products such as beta-cypermethrin, abamectin, chlorbenzuron, matrine, *etc.*) were undertaken during outbreak years in China within the range of the pests. Identification of the sex pheromone of *S. cinerearia* was initiated with the hope that the synthetic pheromone would be of value for population monitoring and for control by mass trapping, especially in the urban area.

In the geometrid moths (Lepidoptera: Geometridae), polyenes and epoxides that comprise the reported pheromone and sex attractant components have been described in detail by Millar (2000). For example, *Abraxas grossulariata* L. (Tóth *et al.*, 1992), *S. sexmaculata* (Packard) (Gries *et al.*, 1993), *Itame occiduaria* (Packard), *I. brunneata* (Thunberg), and *Epelis truncataria* (Walker) (Millar *et al.*, 1990) have exploited (3*Z*, 6*Z*, 9*Z*)-3, 6, 9- heptadecatriene (3*Z*, 6*Z*,

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9Z-17: H) and (6Z, 9Z)-6, 9-*cis*-3, 4-epoxy-heptadecadiene (6Z, 9Z-*cis*-3,4-epoxy-17: H) as pheromone or sex attractant blends. Other species responding to (3Z, 9Z)-3, 9-*cis*-6, 7-epoxy-heptadecadiene (3Z, 9Z-*cis*-6,7-epoxy-17: H) and 3Z, 6Z, 9Z-17: H include *S. ulsterata* (Pearsall) and *S. signaria dispuncta* (Walker) (Millar *et al.*, 1990). These species can produce and respond to a single enantiomer with the other enantiomer being inactive or antagonistic. In addition, the enantiomers and regioisomers are critical to maintain the reproductive isolation between sympatric species.

As for *S. cinerearia*, the biological studies on the sex pheromone were initiated by Ren *et al.* (1991), and the location and structure of the sex pheromone glands in the female moths were elucidated through the electroantennogram (EAG) technique, scanning electromicroscopy (SEM) and histological examination. An extract from the female sex glands of *S. cinerearia* attracted conspecific males in field tests. Subsequently, a major EAG-active component was isolated from the extract and identified by GC-MS, GC-infrared (IR) and microchemical reactions as 6Z, 9Z-*cis*-3, 4-epoxy-17: H. Another minor yet important component was also identified as 3Z, 6Z, 9Z-17: H (Li *et al.*, 1988, 1993). The racemic monoepoxydienes were prepared by Sharpless asymmetric epoxidation with propynol and 1-bromoheptane as the starting materials (Wang *et al.*, 1992) and 3Z, 6Z, 9Z-triene was synthesized with the propargyl reaction of terminal kyne under phase transfer condition in the presence of Cu(I) salt (Gong *et al.*, 2000). All of the epoxides have two enantiomeric forms and determining which enantiomer is produced by *S. cinerearia* can be crucial. The resolutions of some (6Z, 9Z)-6, 9-*cis*-3, 4-epoxide enantiomers have been achieved with custom-made chiral cyclodextrin GC stationary phase (Szöcs *et al.*, 1993; Tóth *et al.*, 1994). In order to develop an effective and nonpolluted way to monitor and control *S. cinerearia*, we defined the optimized conditions for the resolution of the enantiomers of 6Z, 9Z-*cis*-3, 4-epoxy-17: H by CycloSil-B GC stationary phase.

2 MATERIALS AND METHODS

2.1 Insect and pheromone extraction

Larvae of *S. cinerearia* at the 5th or 6th instar were collected in the western suburbs of Beijing in June 2010, and reared with *S. japonica* leaves at room temperature. The pupae were sexed, and the female pupae were kept at 20 – 22℃ with a reversed photoperiod of 16∶8 light∶dark cycle. The 2 – 3 d old female moths were used for pheromone extraction at its calling time of 6 – 8 h of the scotophase. Pheromone glands were dissected and extracted with distilled hexane for 30 min in a capillary glass tube.

2.2 Chemicals

3Z, 6Z, 9Z-17: H, 6Z, 9Z-3R, 4S-epoxy-17: H, and 6Z, 9Z-3S, 4R-epoxy-17: H were obtained from Dr. J. G. Millar (University of California, Riverside, USA). Chemical purity of 3Z, 6Z, 9Z-17: H was determined to be 97% with a BP-20 column, and purities of 6Z, 9Z-3S, 4R-epoxy-17: H and 6Z, 9Z-3R, 4S-epoxy-17: H were determined to be 72% and 73%, respectively. Analyzed with the CycloSil-B chiral capillary column, the enatiomeric purities of 6Z, 9Z-3R, 4S-epoxy-17: H and 6Z, 9Z-3S, 4R-epoxy-17: H were 71.6% enantiomeric excess (ee) and 72.2% ee, respectively.

2.3 GC and GC-MS analyses

Extracts and reference compounds were analyzed on a Hewlett-Packard 5890 Series Ⅱ GC (in splitless mode), equipped with a flame ionization detector (FID) and a BP-20 column (50 m × 0.25 mm I. D., 0.25 μm film, Scientific Glass Engineering Pty. Ltd., Australia). The oven temperature was programmed as follows: the initial temperature was 80℃, raised to 210℃ at 10℃/min, and then held at this temperature for 30 min. Nitrogen was used as carrier gas at column head pressure of 175 kPa.

The absolute configuration of gland component, 6Z, 9Z-*cis*-epoxy-17: H was determined using a chiral capillary column CycloSil-B [30% heptakis (2, 3-di-O-methyl-6-O-t-butyl dimethylsilyl)-β-cyclodextrin in DB-1701; 30 m × 0.25 mm I. D. × 0.25 μm film, Agilent Technologies]. To achieve a better separation of the 3R, 4S- and 3S, 4R-monoepoxydiene enantiomeric isomers, we have carefully optimized the oven temperature. The oven temperature was initially set at 80℃, and then raised to 140℃ at 10℃/min, and isothermal at 140℃ for 170 min. Nitrogen was used as carrier gas at column head pressure of 90 kPa.

GC-MS analyses of pheromone gland extracts and references were performed on HP6890 GC interfaced with a Finnigan Trace DSQ MS (EI mode, 70 eV, mass range 41 – 460 amu) using a 60 m × 0.25 mm I. D. × 0.25 μm film HP-INNOWax column (J&W Scientific, USA) with helium as the carrier gas (1.0 mL/min). The temperature program was the same as that of GC analyses on BP-20 column.

3 RESULTS

3.1 Confirmation of the pheromone gland components

Two components in the female gland extracts of *S. cinerearia* were identified by GC-MS in electron impact (EI) mode. GC/EI-MS spectra revealed the presence of a base peak at *m/z* 79, a large diagnostic ion at *m/z* 108 [$CH_3CH_2(CH=CH)_3H$]$^{+\bullet}$, and a distinct ion at *m/z* 178 ($M^{+\bullet}$-C_4H_8), whereas the peak of molecular ion *m/z* 234 ($M^{+\bullet}$) indicating the presence of 3*Z*, 6*Z*, 9*Z*-17: H, was very weak. Spectrum of 6*Z*, 9*Z*-*cis*-3, 4-epoxy-17: H was characterized by ions at *m/z* 250 ($M^{+\bullet}$), 232 ($M^{+\bullet}$-18), 221($M^{+\bullet}$-29), 203, 192 ($M^{+\bullet}$-58), 178 ($M^{+\bullet}$-72), 59 ($C_3H_7O^+$), and base peak 79. The diagnostic ions and their intensity ratios were in agreement with those of synthetic 3*Z*, 6*Z*, 9*Z*-17: H and 6*Z*, 9*Z*-*cis*-3, 4-epoxy-17: H, respectively. In addition, the two components in the extracts (4 FE) had the same GC retention time as those of synthetic references in both BP-20 and CycloSil-B capillary column (oven temperature program: 80℃ (0 min) – 10℃/min – 210℃ (30 min), no enantiomeric separation occurred). The ratio of 6*Z*, 9*Z*-*cis*-3, 4-epoxy-17: H to 3*Z*, 6*Z*, 9*Z*-17: H was 100∶4.8 ± 1.3 (*N* = 12). Apart from the two components mentioned above, three hydrocarbons, tricosane, pentacosane, and heptacosane with a ratio (based on 6*Z*, 9*Z*-*cis*-3, 4-epoxy-17: H, 100) of (166 ± 166)∶(184 ± 168)∶(307 ± 156) (*N* = 12) were also identified based on the NIST02 MS library.

3.2 Absolute configuration of the gland component, 6*Z*,9*Z*-*cis*-3,4-epoxy-17:H

The absolute configuration of the pheromone gland component, 6*Z*, 9*Z*-*cis*-3, 4-epoxy-17: H was determined by chiral GC using CycloSil-B chiral capillary column. Various temperature programs have been tested for a better separation of the two enantiomers, and finally the temperature program had been optimized to 80℃ – 10℃/min – 140℃ (170 min), at which the two enantiomers 3*R*,4*S*- and 3*S*,4*R*- can be separated efficiently. Under this conditions, the two synthetic enantiomers showed an α value, *i. e.*, t_R(3*S*,4*R*) ∶ t_R(3*R*,4*S*) = 1.015 (Fig. 1: A), indicating that the difference in retention time was larger than 2.2 min between the time range of 146 – 148 min. This separation also enabled us to determine enantiomeric excess (ee) of

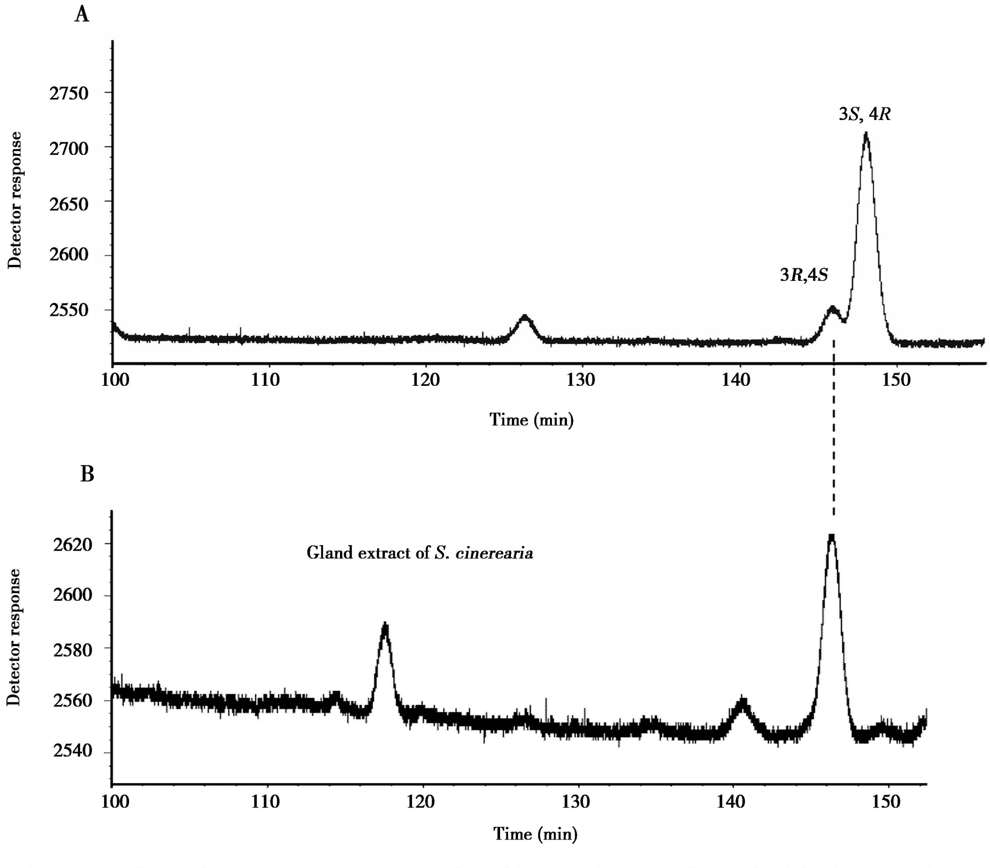

Fig. 1 GC traces of the analyses of synthetic 6*Z*, 9*Z*-3*R*, 4*S*-epoxy-17: H and 6*Z*, 9*Z*-3*S*, 4*R*-epoxy-17: H (A) and the sex pheromone glands of *Semiothisa cinerearia* (B) on CycloSil-B capillary column

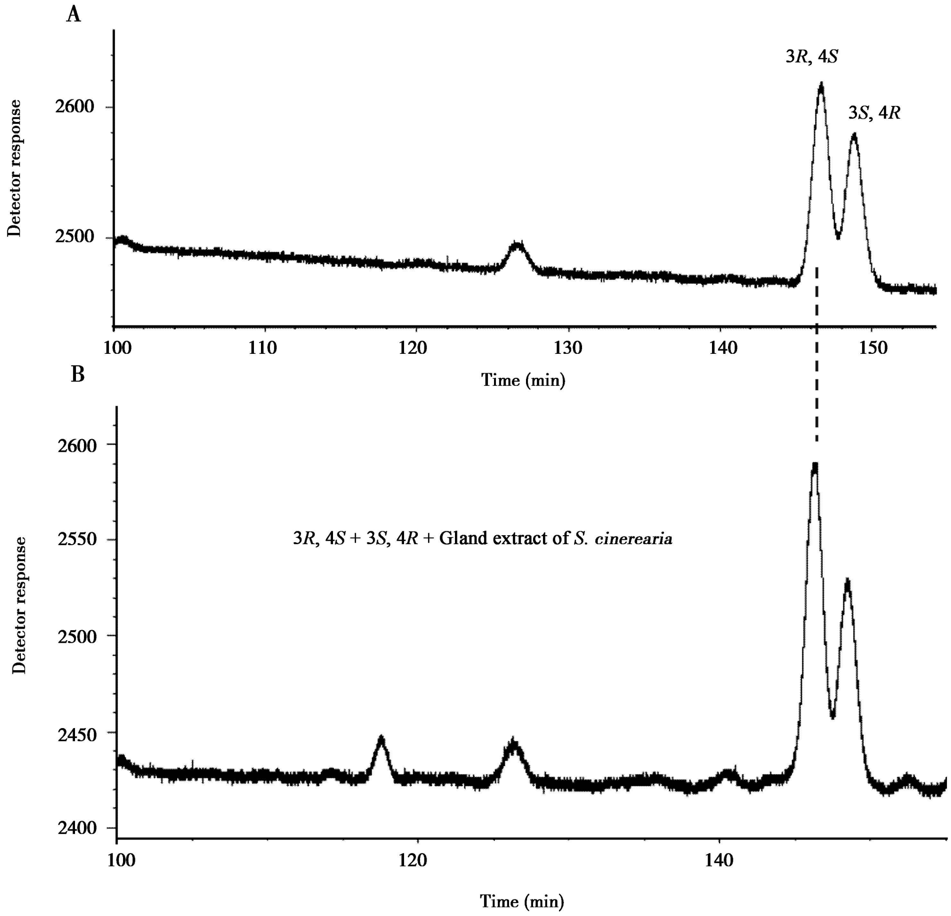

Fig. 2　GC traces of the analyses of synthetic mixture of 6Z, 9Z-3R, 4S-epoxy-17: H and 6Z, 9Z-3S, 4R-epoxy-17: H (A) as well as the two synthetic enantiomers plus the pheromone gland extracts of *Semiothisa cinerearia* (B) on CycloSil-B capillary column

synthetic 6*Z*, 9*Z*-3*R*, 4*S*-epoxy-17: H and 6*Z*, 9*Z*-3*S*, 4*R*-epoxy-17: H as 72%, which is very close to that provided by Dr. J. G. Millar (personal communication, 2004). Under the same condition, the GC retention time of 6*Z*, 9*Z*-*cis*-3, 4-epoxy-17: H (about 4 ng) in the female extracts (3 FE) was 146.271 min (Fig. 1: B), which was very close to that of synthetic 6*Z*, 9*Z*-3*R*, 4*S*-epoxy-17: H (146.235 min) and different from that of synthetic 6*Z*, 9*Z*-3*S*, 4*R*-epoxy-17: H (148.269 min) (Fig. 1: A), confirming the presence of 3*R*, 4*S*-epoxy configuration in the female pheromone gland of *S. cinerearia*. In addition, the 3*S*, 4*R*-epoxy configuration was not found in the female extracts under our GC condition (Fig. 1: B). The ratio of 6*Z*, 9*Z*-3*R*, 4*S*-epoxy-17: H to 6*Z*, 9*Z*-3*S*, 4*R*-epoxy-17: H in their mixture was determined as 1.28∶1 when analyzed on the chiral column (Fig. 2: A). After the mixture of the two enantiomers was injected into the female extracts, this ratio was thus changed to 1.55∶1 (Fig. 2: B). Again, the absolute configuration of the gland component 6*Z*, 9*Z*-*cis*-3, 4-epoxy-17: H was confirmed as 3*R*, 4*S*.

4 DISCUSSION

The 6*Z*, 9*Z*-*cis*-3, 4-epoxy-17: H has been identified from female extracts of *S. cinerearia* (Li *et al.*, 1993), but its biological activity has not been tested in the field. This compound has two enantiomeric forms, of which the one secreted by *S. cinerearia* remains unknown. It is well known that the chirality of epoxide component can be critical. For example, one enantiomer could be highly attractive and the other strongly antagonistic. Furthermore, it is difficult to determine the chirality of the monoepoxydiene in gland extracts because of their small quantities (Tóth *et al.*, 1992; Gries *et al.*, 1993). The fact that male *S. sexmaculata* were most strongly attracted to enantiomerically enriched 6*Z*, 9*Z*-3*R*, 4*S*-epoxy-17: H (69% ee) suggested the stereochemistry as 3*R*, 4*S* configuration (Gries *et al.*, 1993). In order to identify the absolute configuration of the monoepoxydiene from pheromone glands of *S. cinerearia*, an optimized temperature program has been developed with chiral CycloSil-B

column in this work. Under our GC condition (80℃ to 140℃ at 10℃/min, and isothermal at 140℃ for 170 min), we have demonstrated that the female moth of *S. cinerearia* produces epoxyheptadecadiene compound with 3*R*, 4*S*-configuration.

The minor component 3*Z*, 6*Z*, 9*Z*-17: H was also detected from the crude extract of female glands of *S. cinerearia*, though its biological effect has not yet been evaluated until now. This compound can significantly inhibit the response of male *S. sexmaculata*, and strongly enhance the attraction of sympatric male *S. marmorata* when combined with *R*, *S*-epoxide enantiomer (Gries *et al.*, 1993). Moreover, 3*Z*, 6*Z*, 9*Z*-17: H can synergize attractiveness of male *A. grossulariata* when 10% of this compound was added to 6*Z*, 9*Z*-*cis*-3, 4-epoxy-17: H. As chirally pure monoepoxydienes become available, further investigation can be performed to understand whether 6*Z*, 9*Z*-3*R*, 4*S*-epoxy-17: H serves as a single sex pheromone component in *S. cinerearia* or small amounts of 3*Z*, 6*Z*, 9*Z*-17: H synergize or suppress optimal attraction.

In summary, we determined the 6*Z*, 9*Z*-*cis*-3, 4-epoxy-17: H pheromone of *S. cinerearia* has 3*R*, 4*S*-configuration. The identification of the absolute configuration of the pheromone provides a potential tool for the control of *S. cinerearia* infestation in the urban area. It is anticipated that control of *S. cinerearia* infestation with enantiomerically pure materials will be much more effective.

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槐庶尺蛾性信息素腺体 EAG 活性成分绝对构型的鉴定

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摘要：槐庶尺蛾 *Semiothisa cinerearia* Bremer *et* Grey（鳞翅目：尺蛾科）是我国北方国槐 *Sophora japonica* L. 上的重要食叶害虫。本研究的主要目的是阐明槐庶尺蛾性信息素成分化学结构的绝对构型，为在城市地区环境友好地防控槐庶尺蛾的为害提供一种新方法。经与标准品比较气相色谱保留时间和质谱特征离子，从槐庶尺蛾处女雌蛾（2－3 日龄）性信息素腺体溶剂提取物中检测到顺 6，顺 9-顺-3，4-环氧-十七碳二烯烃和顺 3，顺 6，顺 9-3，6，9-十七碳三烯烃 2 种成分，在腺体中以 100∶4.8 ± 1.3（$N = 12$）的比例存在。槐庶尺蛾性信息素腺体提取物进一步经手性毛细管色谱柱（CycloSil-B，30 m × 0.25 mm × 0.25 μm 液膜厚）分离，在优化的程序升温条件下发现腺体成分顺 6，顺 9-顺-3，4-环氧-十七碳二烯烃具有 3*R*，4*S* 的绝对构型。两种合成的对映异构体混合物顺 6，顺 9-3*R*，4*S*-环氧-十七碳二烯烃和顺 6，顺 9-3*S*，4*R*-环氧-十七碳二烯烃以 1.28∶1 的比例加到腺体提取物中，比例变为 1.55∶1。根据这一分析，腺体成分顺 6，顺 9-顺-3，4-环氧-十七碳二烯烃进一步确认具有 3*R*，4*S* 的绝对构型。该研究结论将为生产上研发高效的槐庶尺蛾性信息素诱芯奠定坚实的基础。

关键词：槐庶尺蛾；性信息素；顺 6，顺 9-3*R*，4*S*-环氧-十七碳二烯烃；绝对构型；气相色谱-质谱；手性分离

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